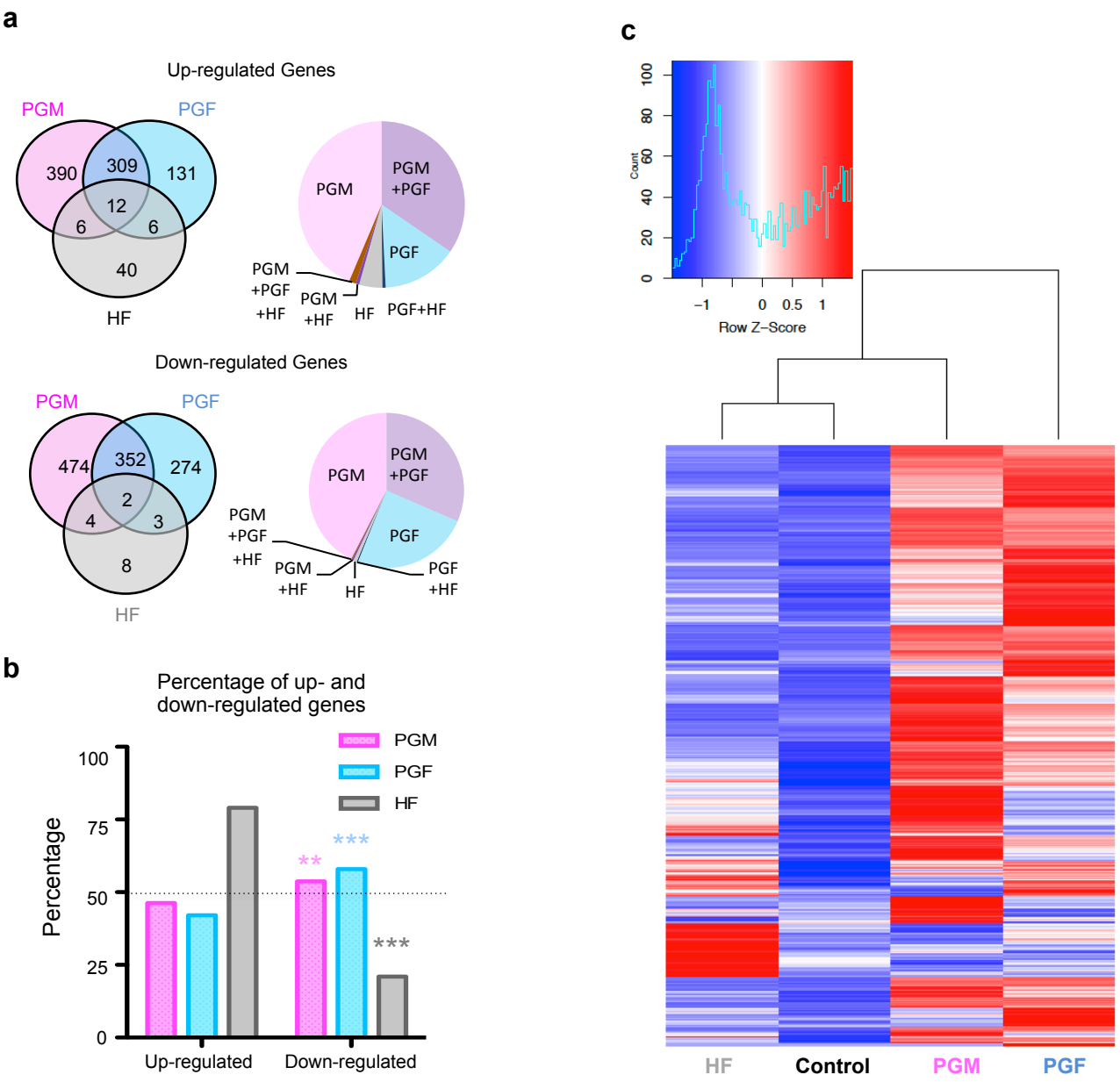
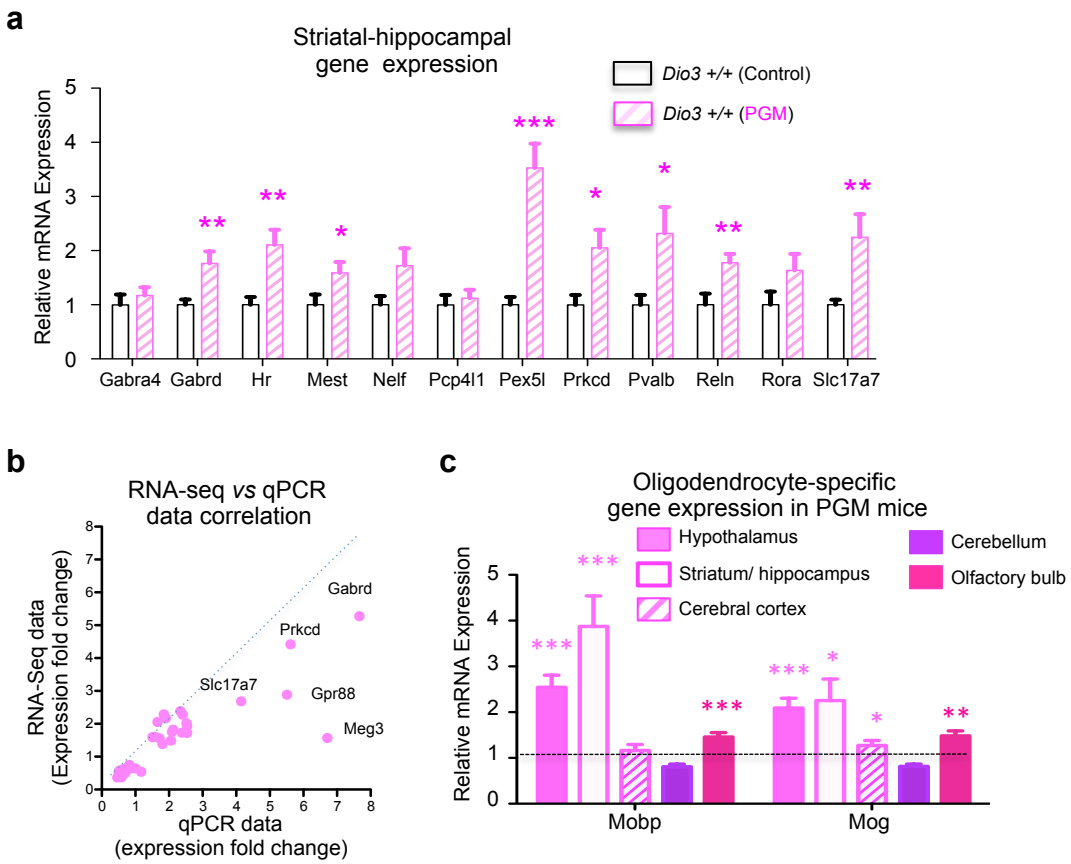
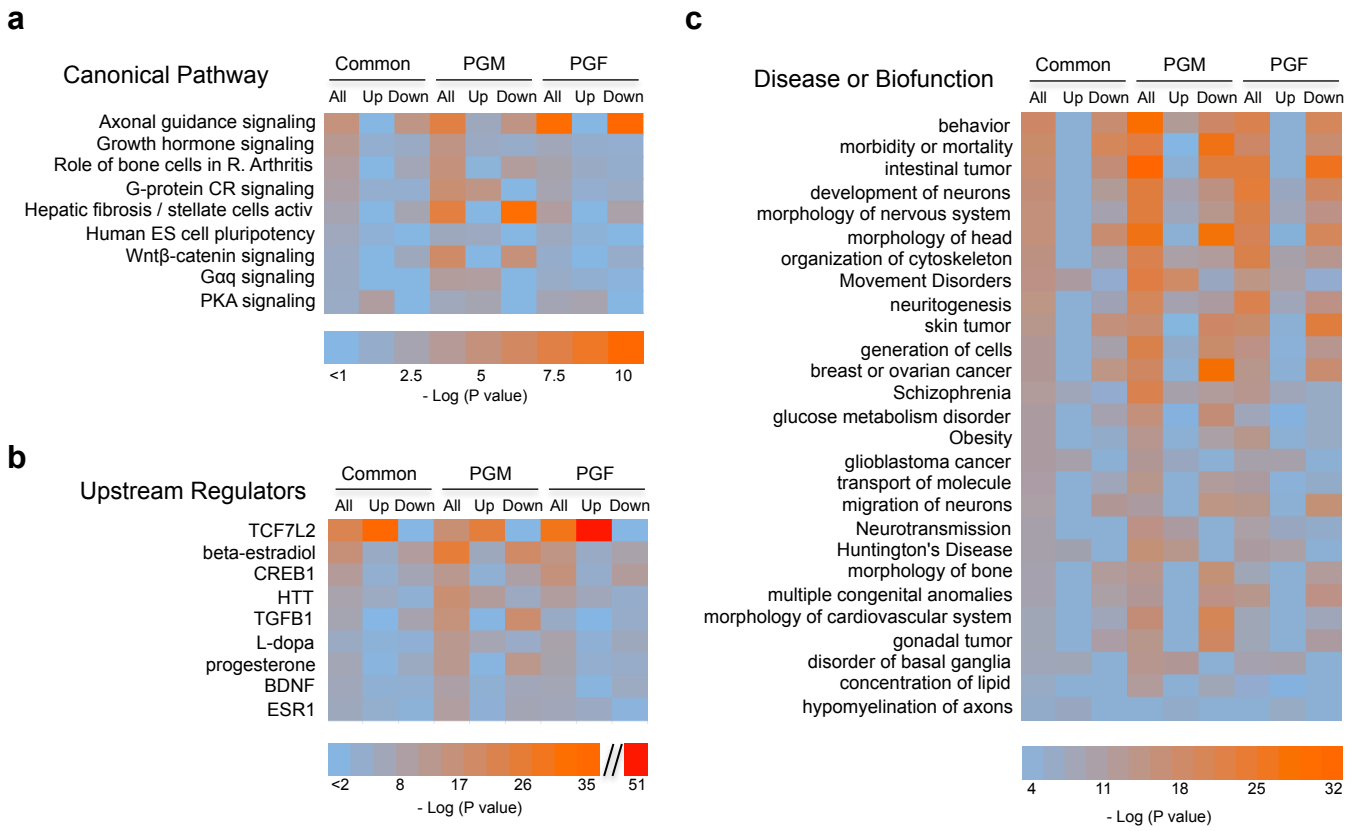


Supplementary Fig. 1. Differentially expressed genes in the hypothalamus of mice descendants from T3-overexposed ancestors. (a), Distribution of up-regulated and down-regulated genes across experimental groups; **(b)** Percentage of upregulated and down-regulated genes in each group; **(c)** Clustering and heat map of all differentially expressed genes across experimental groups.

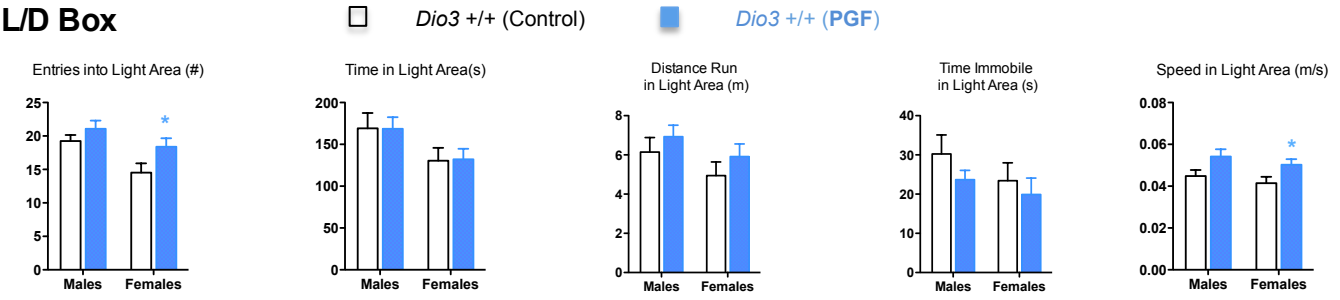


Supplementary Fig. 2. Expression of genes identified as differentially expressed in the hypothalamus of mice descendants from T3-overexposed ancestors. (a) Expression in the P15 striatum-hippocampus of PGM mice; **(b)** correlation of the expression fold change between RNA-sequencing nd qPCR data for 32 genes; **(c)** Expression of two oligodendrocyte-specific, myelin-related genes in different areas of the brain of P15 PGM mice.

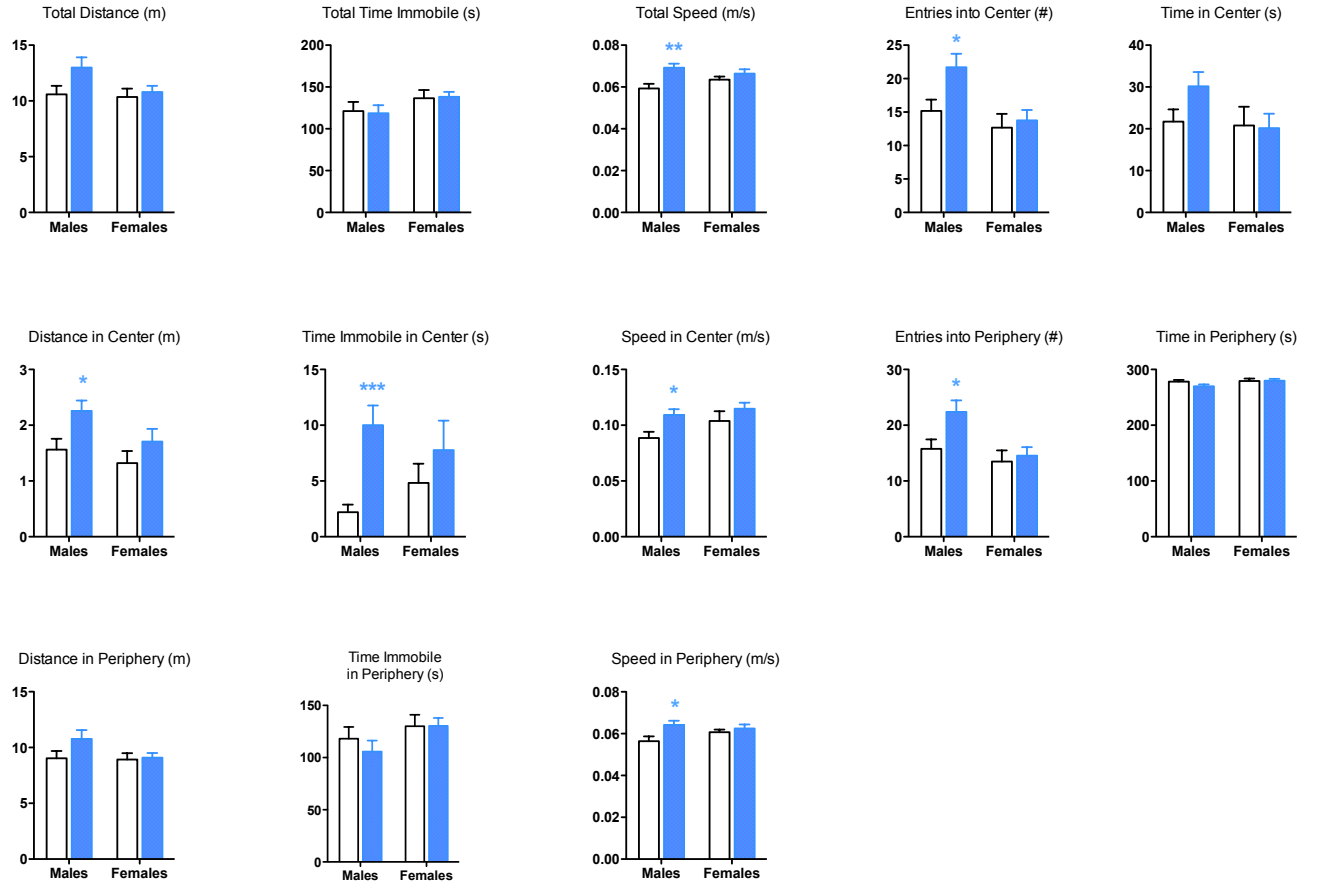


Supplementary Fig. 3. Extended INGENUITY analysis of differentially expressed genes in the hypothalamus of mice descendants from T3-overexposed ancestors. (a) Canonical pathways; **(b)** Upstream Regulators; **(c)** Disease or biofunction.

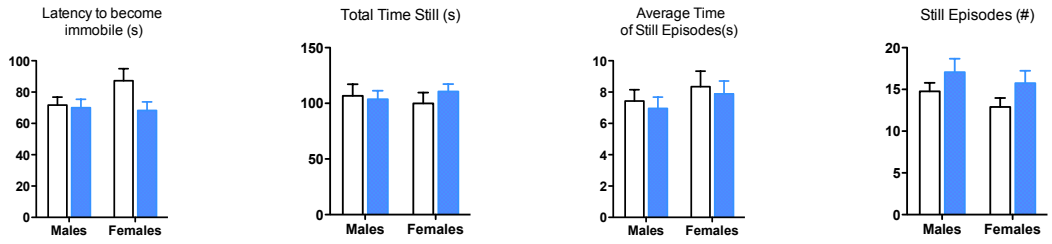
L/D Box



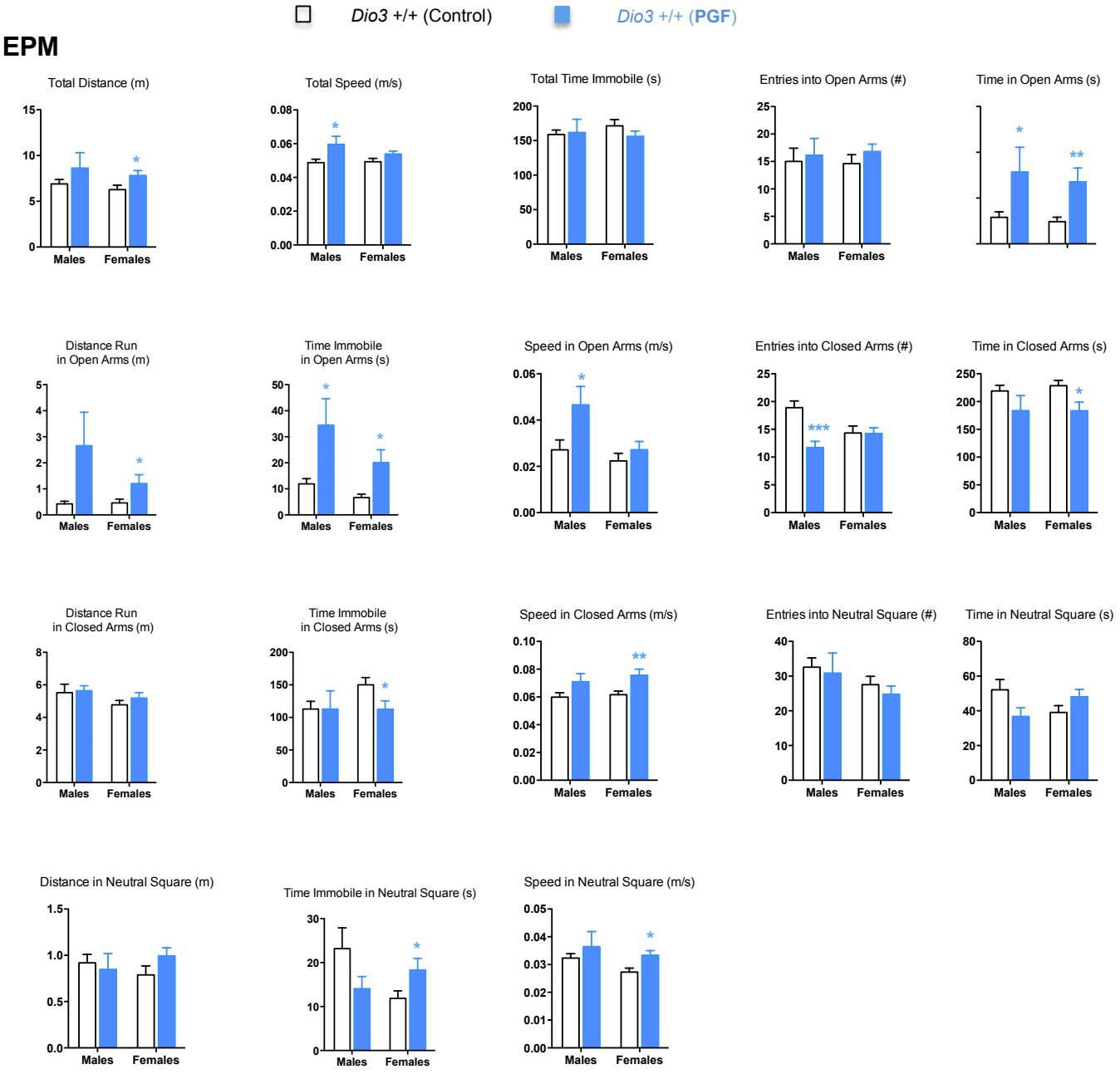
OFT



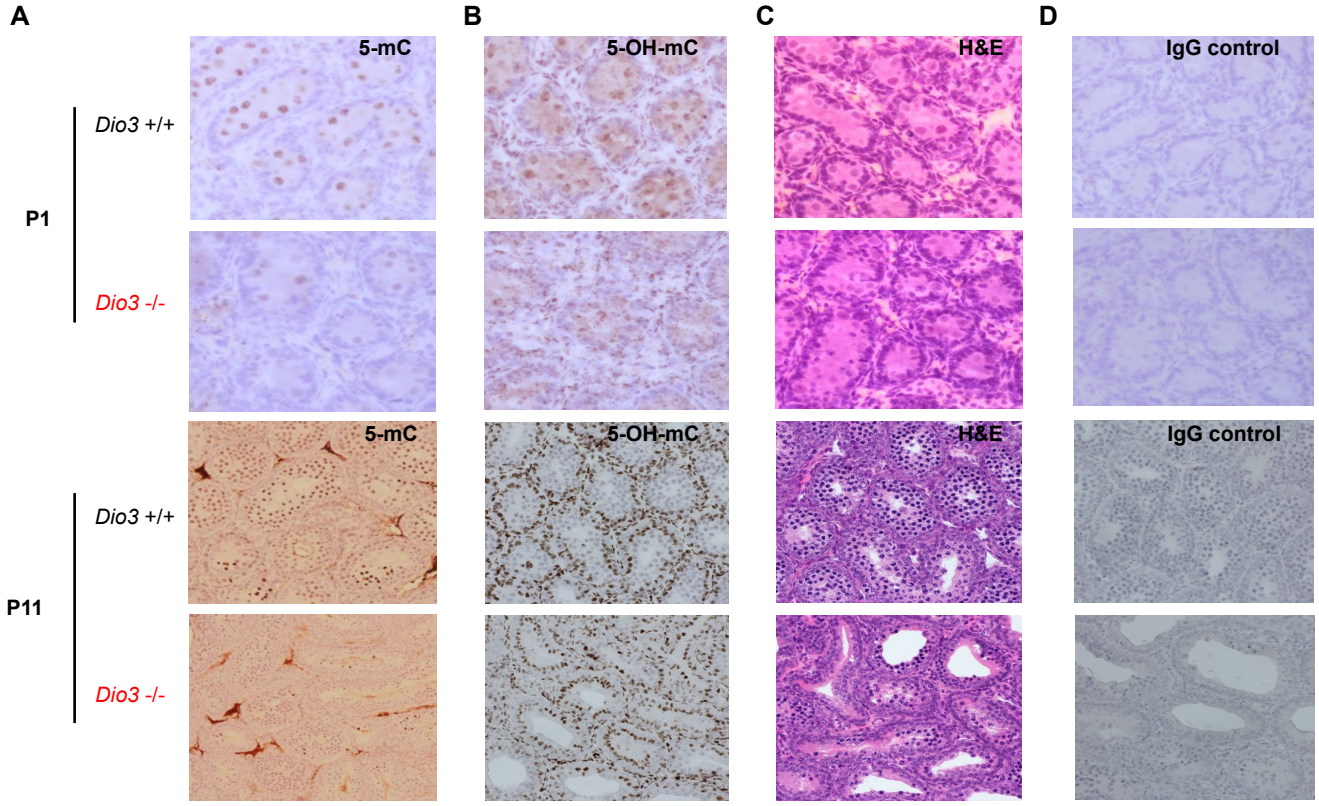
Tail Suspension



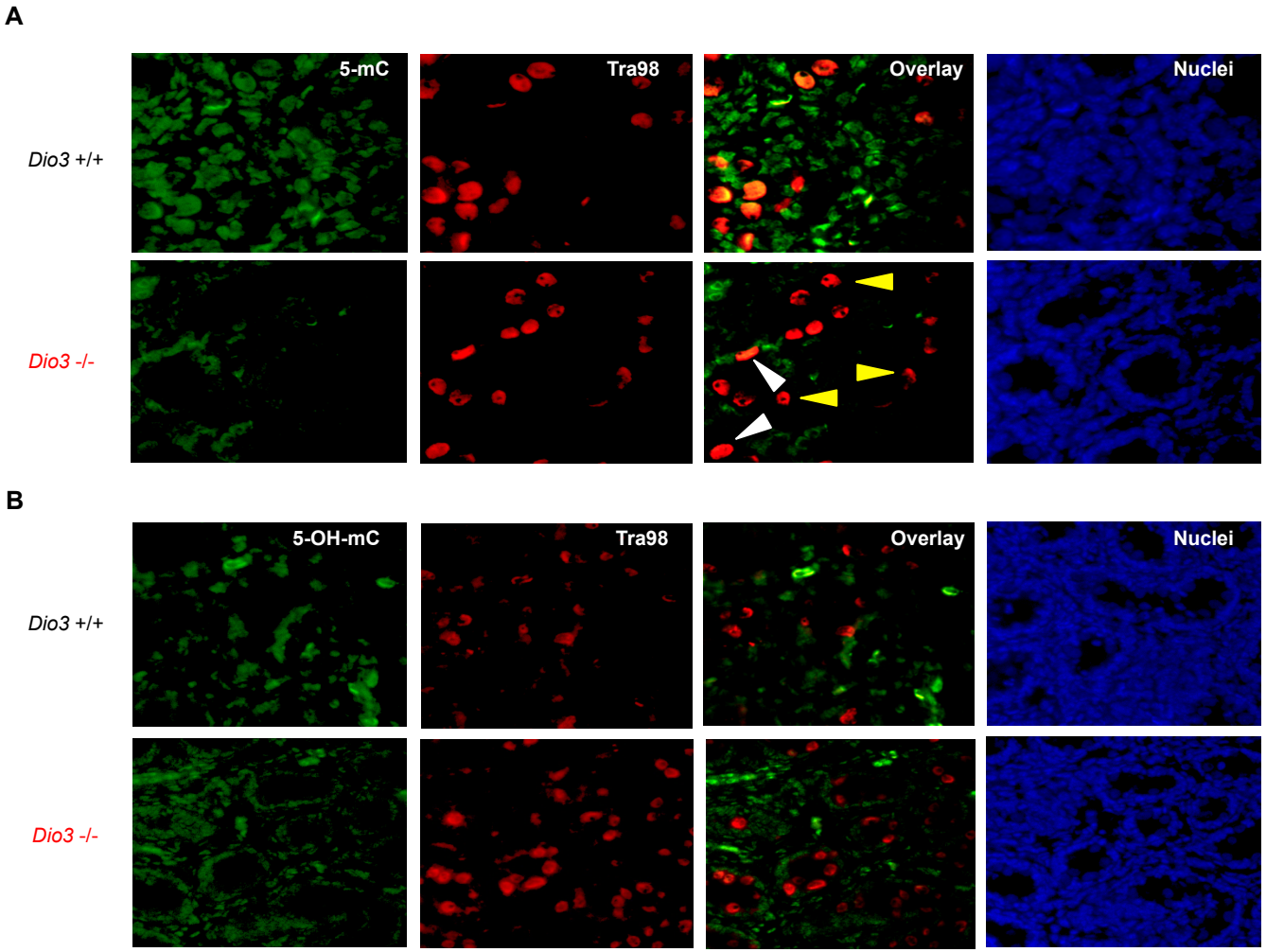
Supplementary Fig. 4. Behavior in adult PGF mice. Parameters measured in the light / dark box test (top), in the open field test (middle) and in the tail suspecnsion test (bottom. Data represent the mean ± SEM of 11 to 16 mice born to first pregnancies from three to five different females. ***, ** and * indicate P <0.001, P<0.01 and P<0.05, respectively, as determined by the Student's t-test, control versus PGF mice.



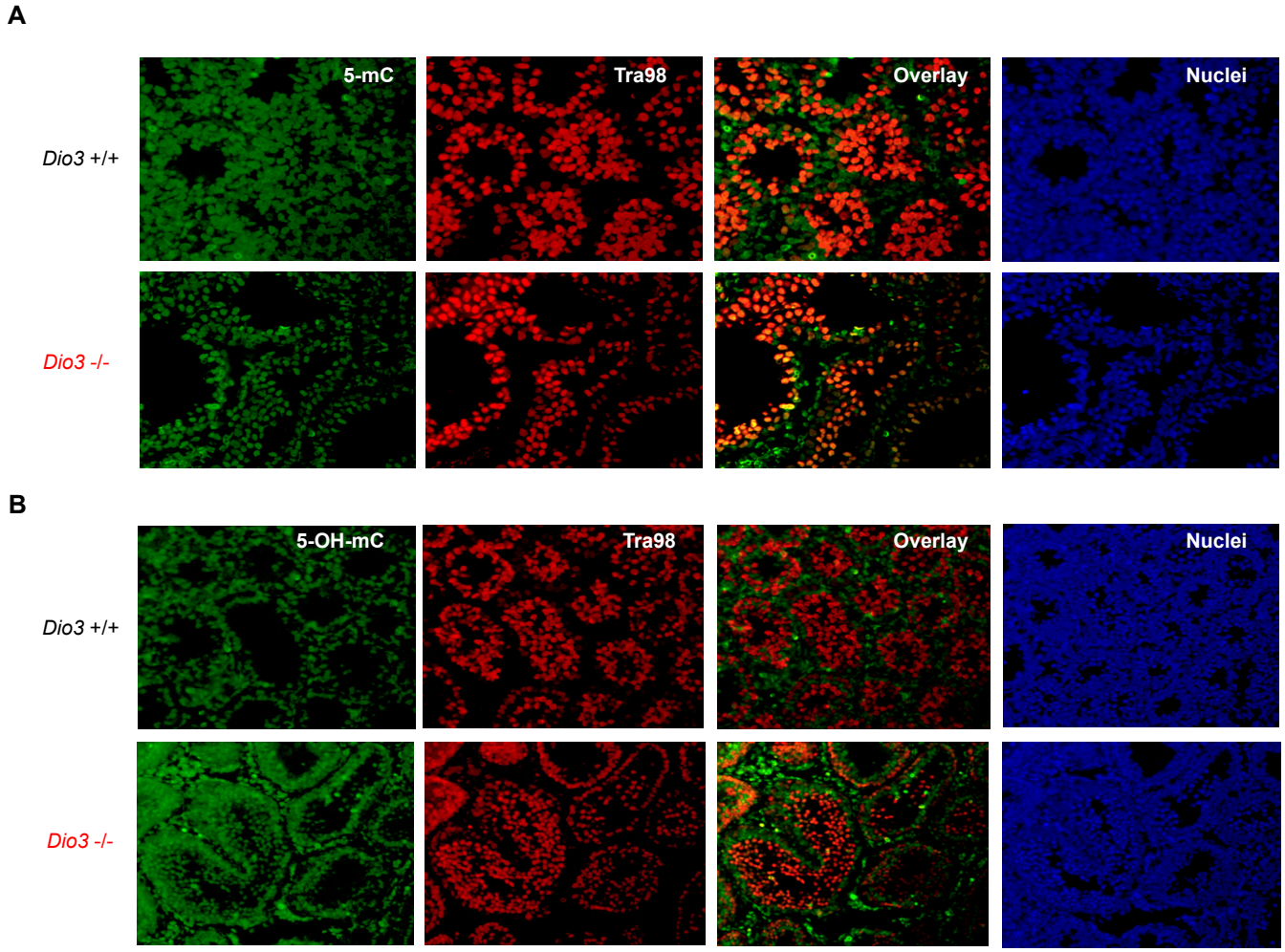
Supplementary Fig. 5. Behavior in adult PGF mice. Parameters measured in the elevated plus maze test. Data represent the mean ± SEM of 11 to 16 mice born to first pregnancies from three to five different females. ***, ** and * indicate P <0.001, P<0.01 and P<0.05, respectively, as determined by the Student's t-test, control vs PGF mice.



Supplementary Fig. 6. **Altered DNA methylation pattern in testes of F0 generation *Dio3 -/-* mice. (a)**, 5-methyl-cytosine IHC staining in postnatal day 1 (P1) and 11 (P11) testis *of Dio3 +/+ and Dio3 -/-* mice. A decrease in 5 mCystaining in *Dio3 -/- mice* is observed at both ages. **(b)**, 5-hydroxy-methyl-cytosine immunohistochemistry staining in postnatal day 1 (P1) and 11 (P11) testis of *Dio3 +/+* and *Dio3 -/-* mice. At both ages, staining in in *Dio3 -/-* testis is decreased and less defined in particular cell nuclei. **(c)**, Hematoxylin and Eosin (H&E) staining. **(d)**, IgG negative control.

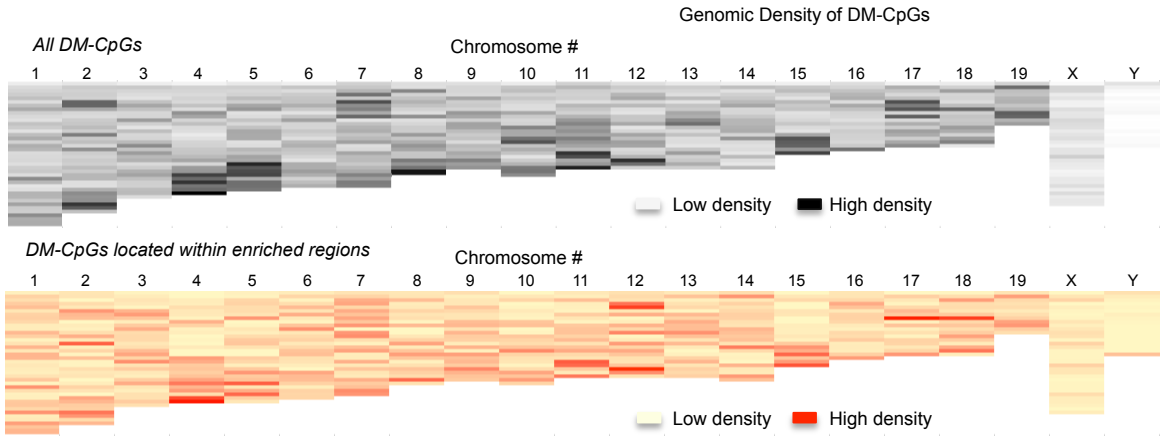


Supplementary Fig. 7. **Altered DNA methylation pattern in male germ cells of 1 day old of F0 generation *Dio3 -/-* mice. (a)**, 5-methyl-cytosine (5mC) immunofluorescence in postnatal day 1 (P1) testis *of Dio3 +/+ and Dio3 -/-* mice. A marked decrease in 5-mC immunofluorescence (green) is observed in *Dio3 -/-* mice. In *Dio3 +/+* mice, germ cell-specific expression (Tra98, red) overlaps with 5-mC immunofluorescence (green). In *Dio3 -/-* mice, while some germ cells (red) exhibit 5mC immunofluorescence (white arrows), a significant percentage of germ cell (red) do not show 5mC immunofluorescence (yellow arrows). This suggests that the marked reduction 5-mC epigenetic marks that is observed in *Dio3 -/-* is affecting a significant subpopulation of germ cells. **(b)**, 5-hydroxy-methyl-cytosine immunofluorescence does not significantly co-localize with germ cells at this age in any genotype.

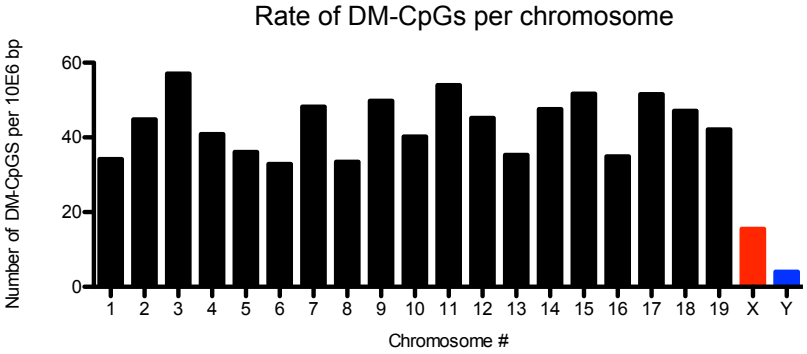


Supplementary Fig. 8. **Altered DNA methylation pattern in male germ cells of 11 day old of F0 generation *Dio3 -/-* mice. (a)**, 5-methyl-cytosine (5mC) IF in postnatal day 11 (P11) testis *of Dio3 +/+ and Dio3 -/-* mice. A decrease in 5-mC IF (green) is observed in *Dio3 -/-* mice. A large proportion of these cells are germ cells (red), as there is overlapping of signals (orange and yellow in overlay). These suggest a reduction of 5-mC epigenetic marks in the DNA of *Dio3 -/-* germ cells. **(b)**, In normal P11 testis, 5-hydroxy-methyl-cytosine IF (green) does not significantly co-localize with germ cells (red), which are neatly organized in the center of developing seminiferous tubules. However, in *Dio3 -/-* mice, germ cells appear less organized and there is a considerable overlap of green and red IF. These results indicates that the germ cells of *Dio3 -/-* mice exhibit an increase in 5-OH-mC, which is consider a first step marker of de-methylation. They also suggest that the structural abnormalities previously described in the testis of *Dio3 -/-* mice [20] include an abnormal distribution of germ cells.

a



b



c

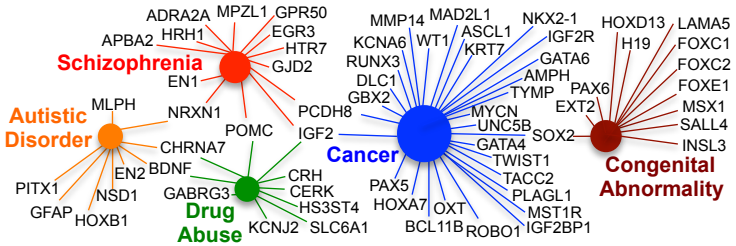
DAVID Analysis

GO Term	FDR
Transcription factor activity	7.2E-32
Embryonic morphogenesis	2.1E-22
Neuron differentiation	2.2E-17

SP-PIR KEYWORD	FDR
Transcriptional Regulation	3.0E-20
Homeobox	1.2E-27
DNA Binding	1.6E-13

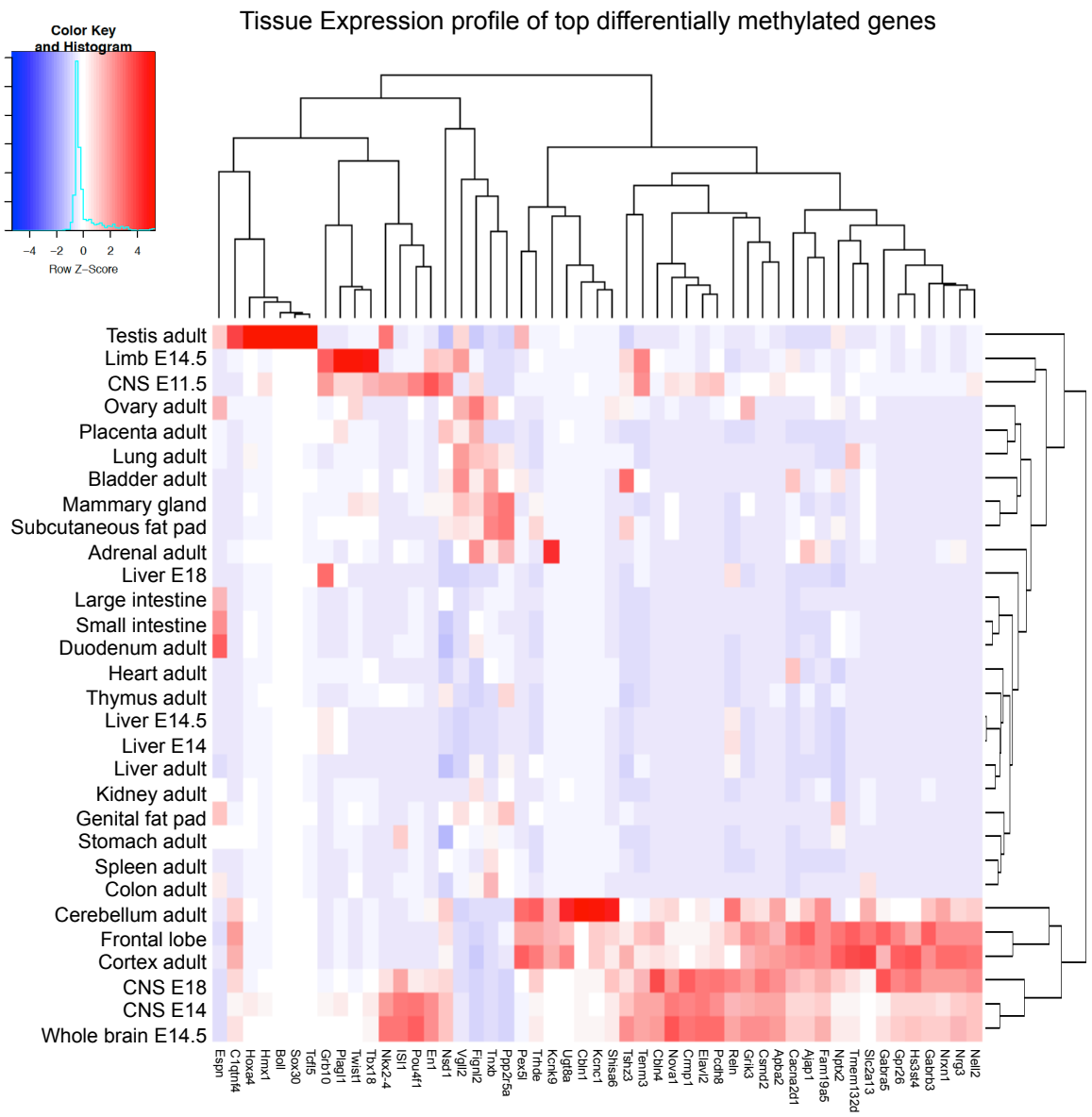
d

FunDO Analysis

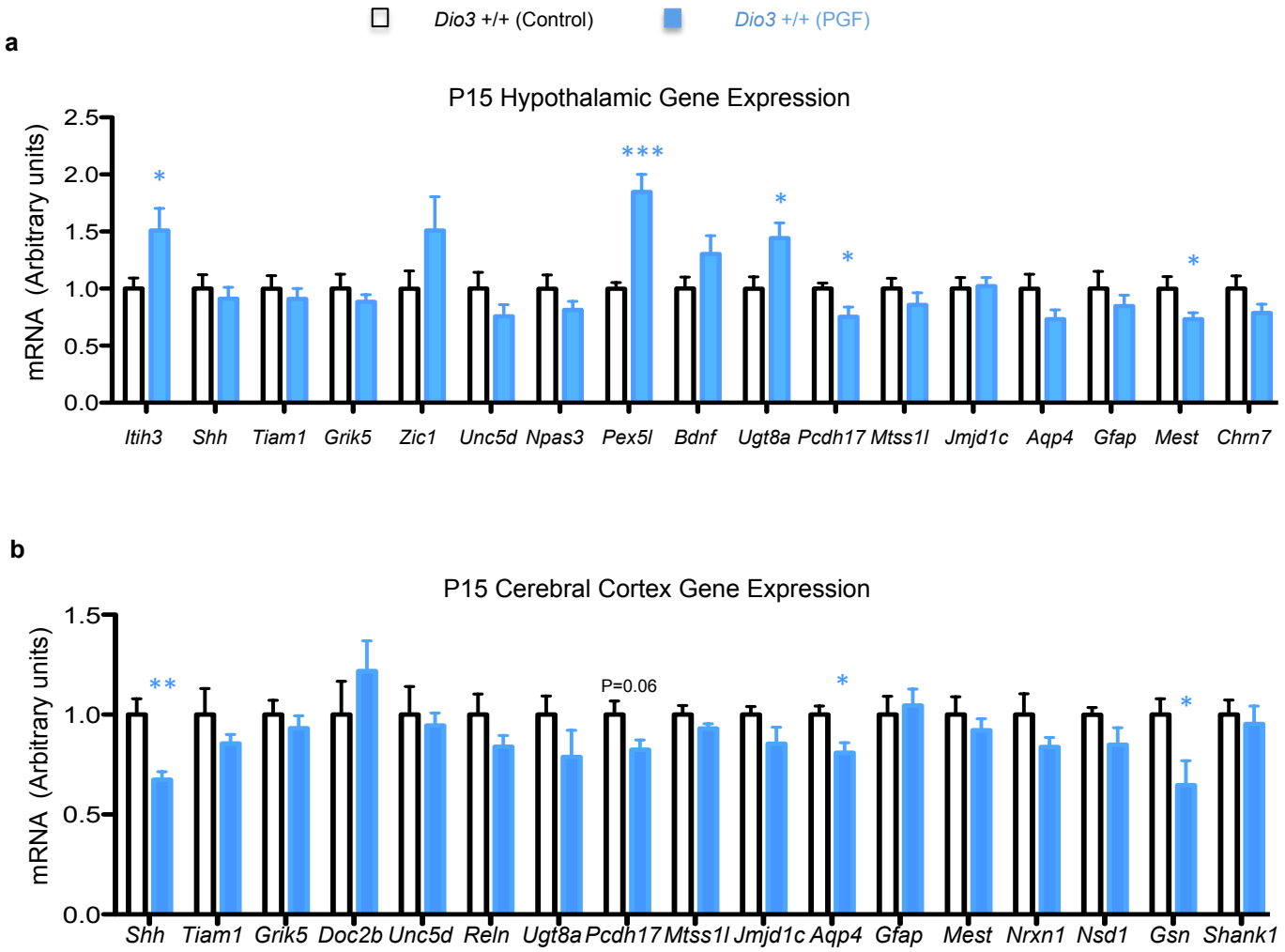


Disease Term	Bonferroni's Adjusted P
Autistic Disorder	8.1E-5
Cancer	9.2E-5
Schizophrenia	2.3E-4
Drug Abuse	8.9E-4
Congenital Abnormality	1.5E-3

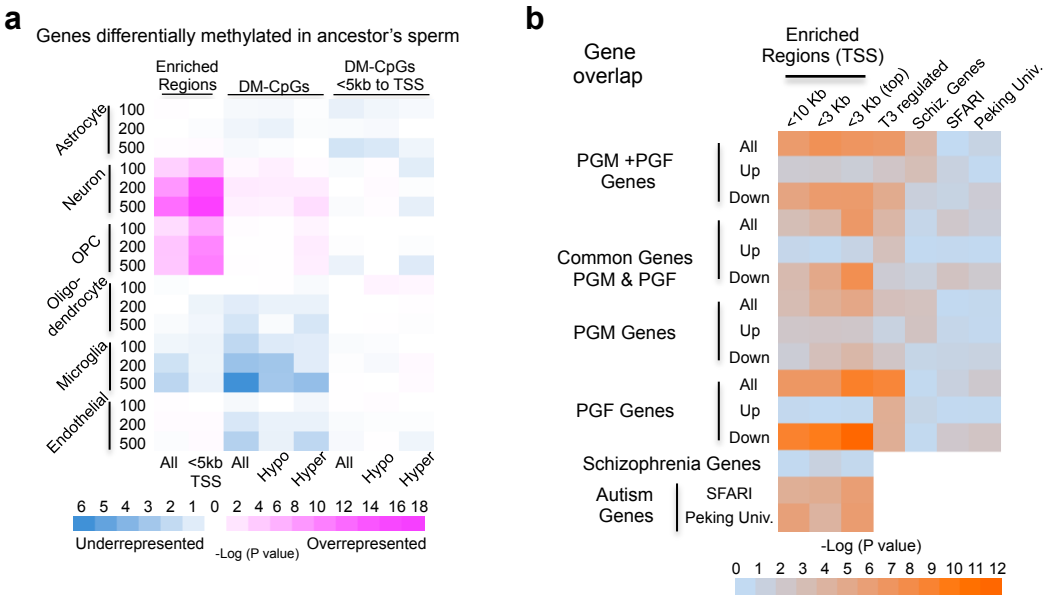
Supplementary Fig. 9. Chromosomal distribution of differential methylation and functional and disease analysis of affected genes. **(a)** Relative genomic densities of all DM-CpGs (top) and DM-CpGs located within enriched regions (bottom) by chromosome**. (b)**, Frequency of Dm-CpGs per chromosome **; (c and d)** DAVID and functional and disease ontology (FunDO) analysis of 597 genes with top enrichment in DM-CpGs with higher than 10% methylation difference less than 8 kb from transcriptional start sites. FDR, false discovery rate.



Supplementary Fig. 10. Tissue expression profiles of top hypomethylated genes. Heat map represents the tissue expression profile of 51 genes (columns) that exhibit the highest number of DM-CpGs within 1kb of the TSS. The expression profile is based on data from the mouse ENCODE transcriptome project available at ncbi/gene website (see Methods). The clustering and profile of most genes indicates high specificity for expression in the adult and developing central nervous system. (Five genes, *Foxc2, Fzd10, Insm2, Klf14 and Rbmxl2*, were not included as no ENCODE expression data was available).



Supplementary Fig. 11. **Brain expression in P15 PGF mice of genes hypomethylated in the sperm of T3-exposed ancestors . (a)**, Gene expression in the P15 hypothalamus. **(b)**, Gene expression in the P15 cerebral cortex. Data represent the mean ± SEM of 7 to 11 mice of either sex born to first pregnancies from two different females. ***, ** and * indicate P <0.001, P<0.01 and P<0.05, respectively, as determined by the Student's t-test.



Supplementary Fig. 12. **Differentially methylated genes in ancestors' sperm. (a)** Enrichment in genes with expression specific to particular brain cells**.** Genes with specific expression in neurons and OPCs are particularly overrepresented among those genes associated with differentially methylated CpG islands in ancestors' sperm. **(b)** Overlap of genes differentially methylated in ancestors' sperm with with those differentially expressed in the F2 generation, and with published compendia of T3-regulated genes in the brain, candidate genes for autism and schizophrenia (see Supplementary Methods). Genes down-regulated in the brain of F2 generation descendants and, more modestly, autism candidate genes, are overrepresented among genes associated with differentially methylated CpG islands in ancestors' sperm.